MICROCHEM LABORATORY

STUDY REPORT

Study Title

Antibacterial Activity and Efficacy of PolyOne Non-porous Test Substance

Test Method

Japanese Industrial Standard Z 2801
Antibacterial Products – Test for Antibacterial Activity and Efficacy

Study Identification Number

NG8195

Study Sponsor

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Test Facility

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Testing performed by: M. Cash

JIS Z 2801: General Information

The Japanese Industrial Standard Committee (JIS) is an international organization that develops and standardizes test methods for a variety of products and materials. The JIS method Z 2801 is a quantitative test designed to assess the performance of antimicrobial finishes on hard, non-porous surfaces. The method can be conducted using contact times ranging from ten minutes up to 24 hours. For a JIS Z 2801 test, non-antimicrobial control surfaces are used as the baseline for calculations of microbial reduction. The method is versatile and can be used to determine the antimicrobial activity of a diverse array of surfaces including plastics, metals, and ceramics.

Laboratory Qualifications Specific to JIS Z 2801

Microchem Laboratory began conducting the JIS Z 2801 test method in 2007. Since then, the laboratory has performed thousands of JIS Z 2801 tests on a broad array of test substances, against myriad bacteria, fungi, and viruses. The laboratory is skilled with regard to modifications of the method to accommodate customer needs. Every JIS Z 2801 test at Microchem Laboratory is performed in a manner that is appropriate for the test substances submitted by the Study Sponsor, while maintaining the integrity of the study.

Study Timeline

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Test Substance Information

The test substances were received on 06 JAN 2017

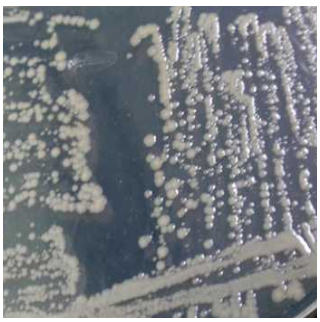
Note: photos depict test substances used in this study

Test Substances Received: PolyOne Control Resin
Withstand™ Ldr: 4.000%
Withstand™ Ldr: 10.000%
Withstand™ Ldr: 25.000%

Test Substances arrived in dimensions that were not optimal for the conduct of the Study. Test substances were cut down to ideal sizes for the Study.

Test Microorganism Information

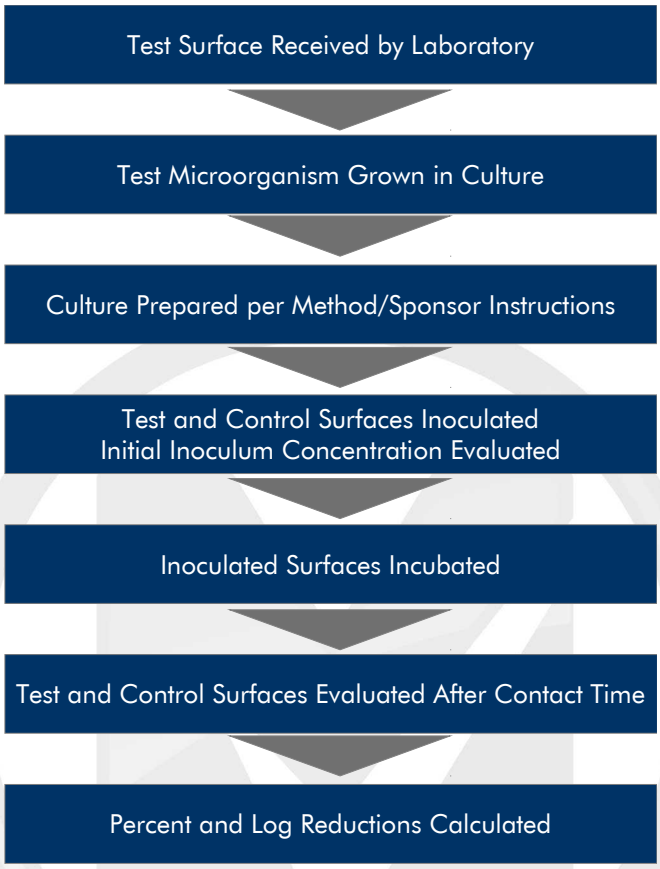
The test microorganism(s) selected for this test:



Escherichia coli 8739

This bacteria is a Gram-negative, rod shaped, facultative anaerobe commonly found in the gastrointestinal tract of mammals. Although most serotypes of this microorganism are harmless there are pathogenic groups of *E. coli* such as enterohemorrhagic (EHEC), verocytotoxin producing (VTEC) and Shiga-like toxin producing (STEC) that can cause a multitude of illnesses. *E. coli* is relatively susceptible to disinfection when dried on a surface, yet it can be a challenging microorganism to mitigate in solution.

Diagram of the Procedure



Summary of the Procedure

- The test microorganism is prepared, usually by growth in a liquid culture medium.
- The suspension of test microorganism is standardized by dilution in a nutritive broth (this affords microorganisms the opportunity to proliferate during the test).
- Control and test surfaces are inoculated with microorganisms, and then the microbial inoculum is covered with a thin, sterile film. Covering the inoculum spreads it, prevents it from evaporating, and ensures close contact with the antimicrobial surface.
- Microbial concentrations are determined at "time zero" by elution followed by dilution and plating to agar.
- A control is run to verify that the neutralization/elution method effectively neutralizes the antimicrobial agent in the antimicrobial surface being tested.
- Inoculated, covered control and antimicrobial test surfaces are allowed to incubate undisturbed in a humid environment for 24 hours, usually at body temperature.
- After incubation, microbial concentrations are determined. Reduction of microorganisms relative to the control surface is calculated.

Criteria for Scientific Defensibility of a JIS Z 2801 Study

For Microchem Laboratory to consider a JIS Z 2801 study to be scientifically defensible, the following criteria must be met:

1. The average number of viable bacteria recovered from the time zero samples must be approximately 1 x 10^4 cells/cm^2 or greater.
2. Ordinary consistency between replicates must be observed for the time zero samples.
3. The number of viable bacteria recovered from the control surface after the contact time must not be significantly (>2-Log_{10}) less than the original inoculum concentration.
4. Positive/Growth controls must demonstrate growth of appropriate test microorganism.
5. Negative/Purity controls must demonstrate no growth of test microorganism.

Passing Criteria

JIS specifies a performance criteria for antimicrobial efficacy of greater than or equal to a 2 Log_{10} or 99% reduction in in the test microorganisms when comparing the treated surface to the control surface after the contact time. Alternatively, passing criteria may be determined by the Study Sponsor in accordance with pertinent governmental regulations.

Testing Parameters used in this Study

Test Substance Size:	45 mm x 60 mm	Film Used? (Size):	Yes (40 mm x 40 mm)
Replicates:	Double (2)		

Culture Growth Media:	Tryptic Soy Broth	Culture Growth Time:	18 hours
Culture Dilution Media:	1:500 Nutrient Broth	Culture Dilution Supplement:	N/A
Inoculum Concentration:	~1 x 10^6 CFU/Sample	Inoculum Volume:	0.400 mL
Contact Time:	24 hours	Contact Temp.:	36°C ± 1°C
Neutralizer:	D/E Broth (10 mL)	Enumeration Plate Media:	Tryptic Soy Agar
Enumeration Plate Incubation Temperature:	36°C ± 1°C	Enumeration Plate Incubation Time:	24-48 hours

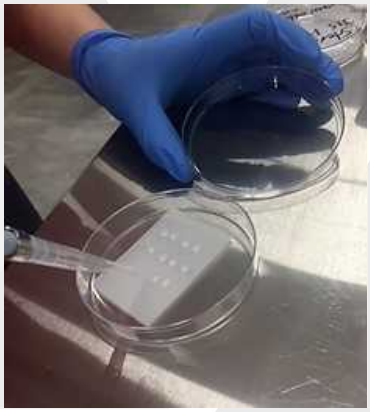
Study Modifications

No further modifications were made to the method for this study.

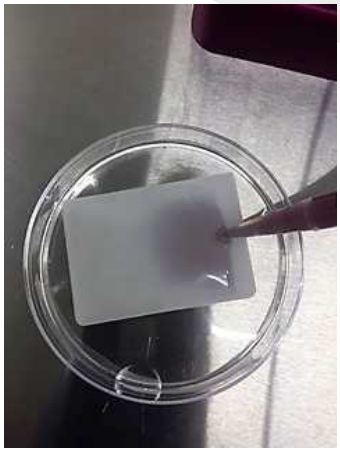
Study Notes

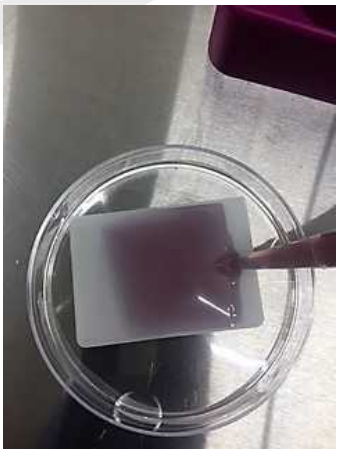
Test substance cut from original size, and measured approximately 45mm x 60mm after cutting. For all test substances, the comparatively thicker side was tested – measuring 0.060" except for PolyOne's control replicate 2. For control replicate 1, 0.060" thickness was tested and for replicate 2, 0.030" thickness was tested.

Study Photographs



Pictured above is the inoculation portion of efficacy testing





The pictures above show the neutralization portion of efficacy testing. Specifically, the neutralization of Withstand™ Ldr: 10.000%

Control Results

Neutralization Method: N/A
Media Sterility: Sterile – No Growth
Growth Confirmation: Confirmed – Target Microorganism

Calculations

$$\text{Percent Reduction} = \left(\frac{B-A}{B}\right) \times 100$$

Where:
B = Number of viable test microorganisms on the control carriers after the contact time
A = Number of viable test microorganisms on the test carriers after the contact time

$$\text{Log}_{10}\text{Reduction} = \text{Log}\left(\frac{B}{A}\right)$$

Where:
B = Number of viable test microorganisms on the control carriers after the contact time
A = Number of viable test microorganisms on the test carriers after the contact time

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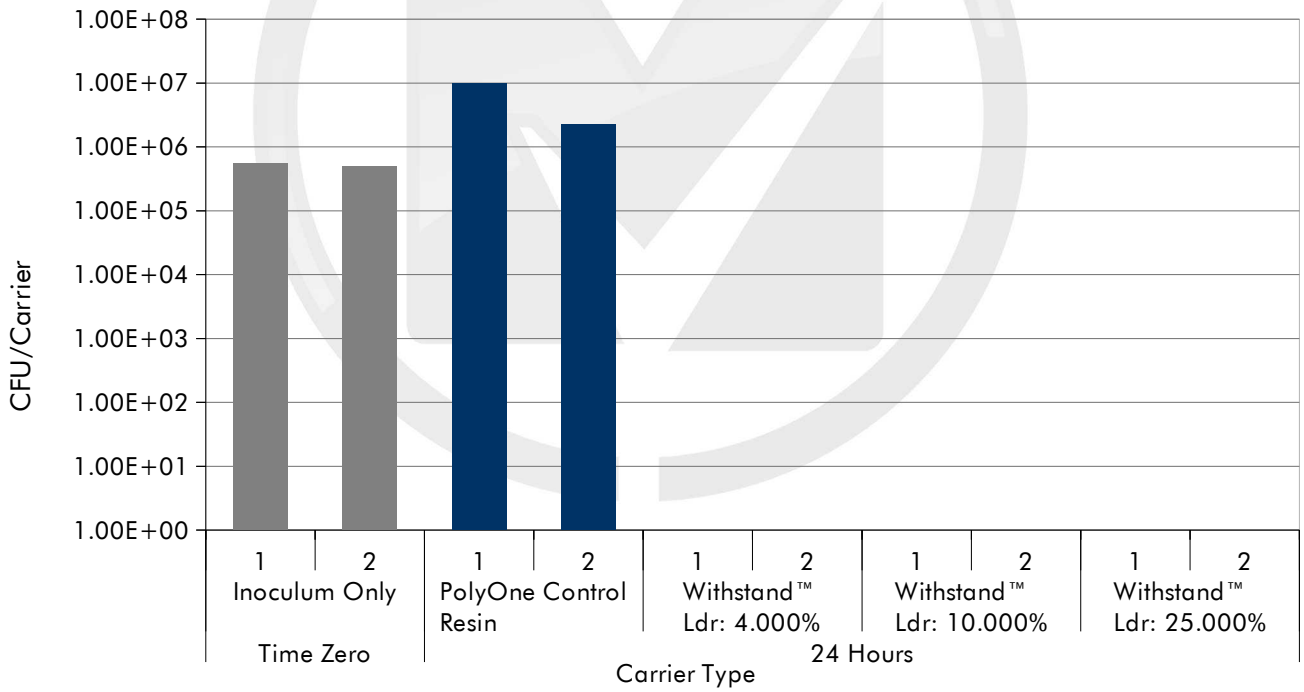
Results of the Study: *E. coli* 8739

Test Microorganism	Contact Time	Carrier Type	Replicate	Replicate CFU/Carrier	Average CFU/Carrier	Percent Reduction Compared to Control at Contact Time	Log_{10} Reduction Compared to Control at Contact Time
E. coli 8739	Time Zero	Inoculum Only	1	5.40E+05	5.15E+05	N/A	
			2	4.90E+05			
	24 Hours	PolyOne Control Resin	1	1.00E+07	6.13E+06		
			2	2.25E+06			
		Withstand™ Ldr: 4.000%	1	<5.00E+00	<5.00E+00	>99.99992%	>6.09
			2	<5.00E+00			
		Withstand™ Ldr: 10.000%	1	<5.00E+00			
			2	<5.00E+00			
		Withstand™ Ldr: 25.000%	1	<5.00E+00			
			2	<5.00E+00			



Note: The limit of detection for this study is 5 CFU/Carrier. Values below the limit of detection are shown as <5.00E+00 on the table and zero on the graph.

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The results of this study apply to the tested substances(s) only. Extrapolation of findings to related materials is the responsibility of the Sponsor.